

Planting Milkweed in the Field Protocol

Notes: The planting site needs to be available for long term data collection (3+ years) and should contain enough space to plant 20 plants 1 meter apart in an appropriate configuration. If you have space and resources, you are welcome to plant additional plots. Please complete a **Plot Data Form** online for each of your plots. If you have multiple plots, you may complete manipulations on some plots, such as spraying with a pesticide to reduce insects. Such an experiment can help us determine the effect of herbivory on plant growth, and to test whether this effect is greater on local or nonlocal genotypes. Although it is ideal if the planting site is the same as the original site of collection, it is more important to choose a site that you can visit regularly to ensure the success of your plants and a site that will allow for data collection annually in the spring and fall. When planting in the field, its best to avoid using soil that already contains milkweed roots. **If your planting site is different from your seed collection site, please complete a second Site Data Form online for the planting site.**

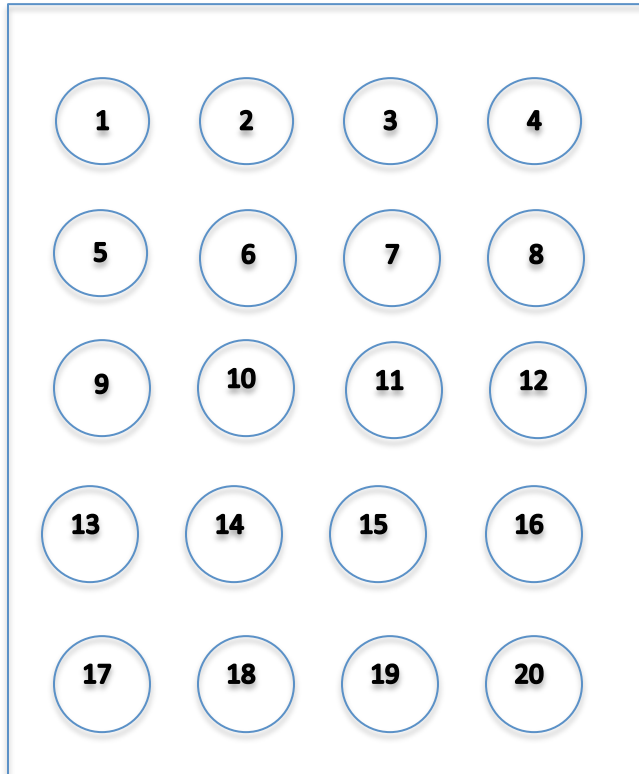
Site Preparation.

1. Existing vegetation should be mowed.
2. We will plant each of the milkweed plants in the field in a pot that serves to contain the roots. This is important for two reasons. First, milkweed plants can spread underground, so the pots will help to ensure that we know which stems belong to each milkweed plant. Secondly, the pots will let us remove the plants from the field when the experiment is finished. Please use **three gallon** pots. They can be ordered from BFG supply or elsewhere; use this [link](#) to see the product.
3. Pots should be positioned 1 meter away from other pots. See the figure below for recommendations about how to set up the plot.
4. Dig holes for the pots, reserving the soil from each hole for the pot.
5. Place the pot in the hole, and fill the pot and area around the pot with the reserved soil. Note that if you have dug up common milkweed plants, you should try to remove the milkweed roots before returning the soil to the pot.
6. *It is best to leave the rim of the pot visible above the soil.* Label the pot with a flag or observable marker to maintain visibility as vegetation returns.
7. Weeds in the pots can cause problems with growth and identification the first year, so we ask that you cover the area within each of the pots with [weed suppression material](#), using staples to affix it to the soil and minimize chances that wind will displace it if necessary. (We cut the material into circles the size of the pot, making a slit to place around the seedling). We will not be covering the material with mulch, and we will remove it at the end of the first growing season.
8. Some sites in our pilot study have experienced high levels of mammalian herbivory that threaten the establishment of experimental seedlings. If this is a problem in your area, we suggest that you establish small chicken wire fences around each pot. Instructions and photos will be forthcoming in a separate document, If you do experience some loss to

herbivory, note that we see many milkweed plants that regrow from their roots later in the season or in a different year.

Randomizing Plants in Field Plots. Similarly to randomizing seedlings, it is important to eliminate the effects of confounding factors such as sunlight and incline on growth patterns between ecotypes. If you are planting multiple plots, repeat this protocol for each plot.

1. For the sake of consistency between projects, we recommend the following layout for your plot. Each number represents a position in your plot.



2. To determine which seedlings to plant in which location, create a data sheet with columns for Plant Source ID number, Replicate, and Random numbers.
3. Fill in the data sheet like the example shown below, except there will be 20 rows in your data sheet. You will have 5 replicate plants of each ecotype in each plot, assuming you had enough seedlings germinate.

Plant.SourceID	Replicate	RandomNumber
1	1	0.061165802
1	2	0.558196935
1	3	0.330180907
1	4	0.75324636
1	5	0.708114391
4	1	0.657579144
4	2	0.074571184

4	3	0.52001157
4	4	0.79148482
4	5	0.766436163
7	1	0.023027252

4. To fill in random numbers, click on the box just below the random number heading. Type in “=RAND().”
5. Hit “enter.”
6. Drag this first box all the way down the data sheet.
7. Highlight **ALL the data in ALL 3** columns of this data table. This is an important step!



8. Go to “Data,” and click on the **Sort** button at the top. You need to do a custom sort so you can tell excel which column to use to sort the data.
9. Under “Column: Sort By,” select “Random Number.” Click “OK.”
10. Your data sheet should now be sorted by the random numbers, with the smallest one at the top.
11. Note that if you click or type in the data sheet, the Random Number column will reshuffle. This is no cause for alarm- the other two columns will not reshuffle.
12. Your table now contains the ecotypes in a random order, which you can use to assign ecotypes to each position in your plot.
13. The example table below is incomplete, but using this example, I would plant ecotype 7 in positions 1 and 2, ecotype 1 in position 3, ecotype 4 in position 4, ecotype 8 in position 5, and so on, for each of the 20 positions in the field plot.

Plant.SourceID	Replicate	RandomNumber
7	1	0.175430329
7	4	0.668760696
1	1	0.153334188
4	2	0.401754989
8	5	0.666883855
9	2	0.923421443
8	3	0.616149693
1	3	0.945682743
9	1	0.73820249
7	3	0.101733135
9	5	0.107602191

14. Repeat the steps above for any extra plots you plant.
15. Make labels for your seedlings that indicate the Plant Source ID, Plot Number, and Position Number to which they are assigned. Its useful to put a label in the seedling pot as well as to create a flag or stick label that can be put in the soil in the field.

16. When it comes time to plant in the field, you will choose five healthy seedlings from each ecotype to plant into the field, retaining extras as backups. To the extent possible, choose a single healthy seedling from 5 separate groups (rather than 5 seedlings from the batch in the same space in you plug tray). Be sure to plant each ecotype in the appropriate field position.

Acclimation to the Outdoors. Transplanting can take a toll on seedlings. Therefore, after growing in a greenhouse or classroom, plants must acclimate to their new outdoor surroundings before being planted in the field. When the danger of frost has past and your seedlings are about 3” tall, acclimate the plants to the outdoors.

1. Water the seedlings well and allow them to begin the process of acclimating, or hardening, to the outdoors by setting them outside in a shady, sheltered area for a while, bringing them back in if they wilt. Beware of wind! Don’t acclimate at times/days when high winds or thunderstorms are expected.
2. Gradually increase the amount of time they are exposed to the outdoors and to sunny, exposed environments, including leaving them outside overnight, as appropriate. This process could take 4-6 days.
3. Continue to water as necessary.
4. It is best to transplant on a cloudy day, if possible.

Transplanting to the Field. In order to perform a common garden study, individuals of the same species but of different ecotypes must be planted together. When multiple common garden studies are performed in different geographic areas corresponding to the sources of the different populations, this is called a reciprocal transplant study. This allows us to determine whether or not a particular species might be locally adapted.

1. Use a flag or other marker to label each pot with the Plot Number, Location Number, and the appropriate Plant Source ID number determined by the randomization process. Do NOT include the Site Names; recall that we are using Plant Source IDs to reduce bias.
2. Cut a hole/slit in the weed suppression material, or temporarily remove it, to allow you to transplant the designated seedling to each buried pot. Dig a hole big enough for the transplant. Reserve the soil to fill in after transplanting.
3. Pour some water into the hole, and then gently transplant the seedling into the hole, taking care to minimize root disturbance. Fill in the hole with soil and water well. Add the weed suppression material back around the transplant, if necessary.
4. After transplanting, take baseline measurements of stem height, stem diameter, total leaf number, node number, and length and width of the largest leaf. For these procedures, please see the “Milkweed Measurement” protocol and supplementary videos.
5. Record these data on the [Baseline Milkweed Measurement Data Form](#). Please submit one form for each plant you transplant.
6. Keep your extra seedlings alive. If any plant in the field dies within **four weeks** of transplanting, replace it with an extra plant from the same ecotype. Please submit a new [Baseline Milkweed Measurement Data Form](#) for replacement transplants.

Emily Mohl
mohl@stolaf.edu; 773-729-0617 (c)

St. Olaf College
1520 St. Olaf Ave. Northfield, MN 55057

7. Water the plants deeply at transplant, and as necessary for the first 3-6 weeks after transplanting to promote establishment. At our sites, we find that rain often provides enough moisture, but we water 2-3 times/week in the absence of rain.
8. If necessary, establish mammal herbivore fence exclosures around each of your transplants to protect them.

Important Note: In the fall, when you measure your plants, please remove weedblock. Your plants should be successfully established at this point.

Contact Emily Mohl at mohl@stolaf.edu or 773-729-0617 with any questions. Thank you!